

Investigation of the Toxic & Teratogenic effects of GRAS Substances to the Developing
Chicken Embryo (**In-house Investigation of Caffeine in the Developing Chicken
Embryo**) 4/10/74

E6

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : Mr. Alan Spiher
GRAS Review Branch, HFF-335

DATE: April 10, 1974

THRU : Dr. Leo Friedman, Director
Division of Toxicology, HFF-150

FROM : M. Jacqueline Verrett, Ph.D. *M. Jacqueline Verrett*
Reproductive Physiology Branch, HFF-157

SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to
the Developing Chicken Embryo.

Attached is the report of the in-house investigations of Caffeine in
the developing chicken embryo.

Investigations of the Toxic and Teratogenic Effects of
GRAS Substances to the Developing Chicken
Embryo: Caffeine

Protocol:

Caffeine (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in tables 1 through 4 for each of the four conditions of test.

Column 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Caffeine showed only slight toxicity when injected into the air cell at either 0 or 96 hours of development. At 0 hours, above 0.125 mg/kg the toxicity increased less than 20 percent above the solvent control, up to the highest level tested of 20 mg/kg. Because of its insolubility in water, this is the highest dose that it was possible to administer. At 96 hours treatment there was no increase in toxicity (above background) between 0.050 and 10.0 mg/kg, and the slope of the line was negative. At 0 hours there was a slight regression of mortality with dose but the slope of the line was not significantly different ($p=0.05$) from zero. (Tables 1 and 2)

Yolk treatment at 0 hours showed high toxicity at all levels between 0.050 and 20 mg/kg but the slope of the line was negative. At 96 hours the solvent background toxicity was high and there was only one dose level that showed significantly higher toxicity (2.50 mg/kg). The slope of the line was negative. (Tables 3 and 4)

Scattered abnormalities were seen under the four conditions of treatment but only a few could be considered serious abnormalities. The incidence of anomalies involving the head, limbs, viscera, and skeleton were low for all modes of treatment. Only with air cell route at 0 hours were two levels seen where this incidence was significantly ($p=0.05$) higher than that of the solvent control: 1.0 mg/kg where there were three birds with serious anomalies, one with buphthalmia and a cleft palate, one with microphthalmia, and one with cryptophthalmia; and 0.10 mg/kg where there was one bird with celosomia. Other abnormalities observed included exencephaly, micromelia, toticollis, brachygnathia, dysgnathia, microblepharia, acrania, and agenesis of the eyelid. Only one bird with each of these abnormalities was seen.

It is concluded that caffeine was not teratogenic to the chicken embryo although some embryotoxicity was observed when it was administered via the yolk before incubation. ✓

1. Caffeine, USP Anhydrous Powder, Lot Q A 70871, Monsanto Chemical Co.
 2. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O.G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770.
 3. Verrett, M.J., Marliac, J.-P., and McLaughlin, J., Jr., (1964) JAOC 47, 1002 - 1006.
 4. Finney, D.J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.
-

Caffeine

Air Cell at 0 Hours

Dose mg/kg	mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal	
				Total	Structural
1.0	20.0	150	36.00*	5.33*	2.00
0.50	10.0	110	30.90	3.63	0.90
0.250	5.0	140	39.28*	4.28*	2.14
0.050	1.0	149	35.57*	4.02	4.02*
0.025	0.50	105	23.80	5.71*	1.90
0.0125	0.250	150	34.66*	1.33	1.33
0.00625	0.125	60	21.66	3.33	0.00
0.0050	0.100	130	25.38	4.61*	3.84*
0.0025	0.050	60	25.00	6.66*	1.66
Water		200	22.50	0.50	0.00
Controls		400	19.75	2.00	1.00

**Slope is not significantly different from zero ($p=0.05$)

* Significantly different from solvent ($p \leq 0.05$)

Caffeine

Air Cell at 96 Hours

Dose mg/kg	mg/kg	Number of Eggs	** Percent Mortality	Percent Abnormal	
				Total	Structural
0.50	10.0	95	13.68	1.05	1.05
0.250	5.0	100	19.00	4.00	2.00
0.125	2.5	90	17.77	5.55	3.33
0.025	0.50	95	14.73	2.10	1.05
0.00625	0.125	95	20.00	4.21	3.15
0.0025	0.050	95	15.78	1.05	0.00
Water		145	22.06	0.68	0.00
Controls		400	19.75	2.00	1.00

**Slope is negative

Table 3

Caffeine
Yolk at 0 Hours

Dose		Number of Eggs	** Percent Mortality	Percent Abnormal	
mg/kg	mg/kg			Total	Structural
1.0	20.0	60	76.66*	1.66	0.00
0.50	10.0	90	64.44*	1.11	1.11
0.250	5.0	60	65.00*	6.66	3.33
0.050	1.0	60	68.33*	0.00	0.00
0.025	0.50	104	79.80*	5.76	0.00
0.0125	0.250	60	61.66*	0.00	0.00
0.00625	0.125	30	73.33*	6.66	0.00
0.0050	0.100	60	66.66*	1.66	0.00
0.0025	0.050	30	86.66*	3.33	0.00
Water		175	26.85	1.71	1.14
Controls		400	19.75	2.00	1.00

**Slope is negative

* Significantly different from solvent ($p \leq 0.05$)

Caffeine

Yolk at 96 Hours

Dose		Number of Eggs	**Percent Mortality	Percent Abnormal	
mg/kg	mg/kg			Total	Structural
0.50	10.0	100	55.00	9.00*	5.00
0.250	5.0	100	49.00	6.00	1.00
0.125	2.50	95	62.10*	5.26	2.10
0.025	0.50	99	53.53	7.07*	5.05
0.00625	0.125	100	33.00	5.00	3.00
0.0025	0.050	100	52.00	6.00	3.00
Water		128	42.18	0.78	0.78
Controls		400	19.75	2.00	1.00

**Slope is Negative

* Significantly different from solvent ($p \leq 0.05$)